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Screening for New Agents

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Screening for new antiviral drugs is concentrated on a search for inhibitors of the human immunodeficiency virus, herpesviruses, influenza virus, hepatitis B virus and rhinovirus. The first step in the process is usually the screening of virus-infected cell cultures followed by secondary screening in infected animals. The relevance of the different screening methods for predicting clinical efficacy is at present uncertain due to the low number of compounds evaluated in double-blind placebo-controlled clinical trials. As a consequence of the considerable activity in ongoing research on antiviral drugs the predictive value of the screening systems is expected to improve.

The identification of compounds which can be used for the treatment and prophylaxis of viral diseases in man is a stepwise procedure. The primary screening is usually performed in cell cultures infected with viruses or in cell-free systems containing viral enzymes. This is followed by secondary screening in different animal models until phase I studies in humans can be started (1-6).

The high cost for pharmaceutical companies of developing a new drug (in the order of US \$ 100-200 million) makes it necessary to limit the search to antiviral drugs for treatment of a few viral diseases. Prime targets are viral diseases which are not easily controlled by vaccination, which affect large numbers of persons in industrialized nations, which can be diagnosed rapidly, and which are preferably severe and require long periods of treatment or prophylaxis. At present the main efforts are directed towards finding treatment for HIV, herpesvirus influenza, hepatitis B and rhinovirus infections. Various aspects of the process of screening for new inhibitors of these viruses as well as some general aspects of screening will be discussed in this paper.

General Aspects of Screening for Antiviral Drugs

For a large number of the compounds available, virus multiplication in cell culture is probably the best primary screening system since it gives the opportunity to identify in one assay inhibitors of all different stages of virus multiplication. If the compounds to be tested are specifically designed to inhibit one viral function, it is probably more appropriate to use this function for screening, since this will yield reliable structure-activity data necessary for the synthesis of better inhibitors.

When using both inhibition of virus multiplication in cell cultures and inhibition of viral enzymes as screening systems, the use of uninfected cells and cellular enzymes are necessary to evaluate non-specific or toxic effects. A therapeutic ratio is obtained by comparing the concentration causing a 50 % inhibition of cell growth or cell enzyme function and the concentration inhibiting viral multiplication or viral enzymes by 50 %. However, this in vitro therapeutic ratio should be used with reservations since, as will be discussed later, it frequently differs considerably from the therapeutic ratio in infected animals and patients.

In the present situation most antiviral drugs have a narrow spectrum of activity which means that model viruses should be avoided if possible and viruses causing human disease preferred. Since isolates differ in sensitivity, it is necessary to test inhibitors, which have been found in screening using one virus isolate, against several

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fresh virus isolates from patients. It is also important to test a newly found inhibitor against virus isolates resistant to other inhibitors.

If possible, human cells should be used in the cell culture screening system. This is especially important when evaluating nucleoside analogs or other compounds transformed by cellular enzymes to the active inhibitor. Species differences in phosphorylation of nucleoside analogs can result in both overestimation and underestimation of antiviral activity. Stationary phase and actively growing cells are also likely to give different results for nucleoside analogs phosphorylated by cellular enzymes since kinase activity will differ. The target cell(s) for virus replication *in vivo* should if possible be used in the screening, but if this is too cumbersome the target cells could be used in a further evaluation once an active compound has been found in a simple primary screening system using other cells.

Cellular toxicity can be determined in many different ways. Determination of the multiplication of cells and their volume during the same time period as used in the assay for antiviral activity would seem to be a reliable method, which is easy to automatise.

In most instances a major task in a primary cell culture screen is the preparation of a large number of solutions with defined concentrations of the different compounds. Antiviral activity and toxicity in cells can often be determined by automated methods with a high capacity but automated methods for processing large number of compounds are not available.

Secondary screening in animal models is a necessary step between cell cultures and trials in humans. The validity of different animal models can only be determined by evaluation of antiviral activity in patients. Unfortunately, very few antiviral drugs have been evaluated in double-blind placebo-controlled clinical trials and animal models, and the predictive value of different animal models is thus uncertain. This situation is likely to improve considerably in the next few years as a result of the extensive research programme on antiviral drugs initiated by the AIDS epidemic.

Rhinoviruses

Rhinovirus enzymes such as RNA polymerase and protease do not seem to have been used for primary screening to any large extent. Most screening for inhibitors of rhinoviruses has been done in cell cultures using human cell-lines (7). There is no satisfactory animal model of human rhinovirus infection, but the mild disease caused

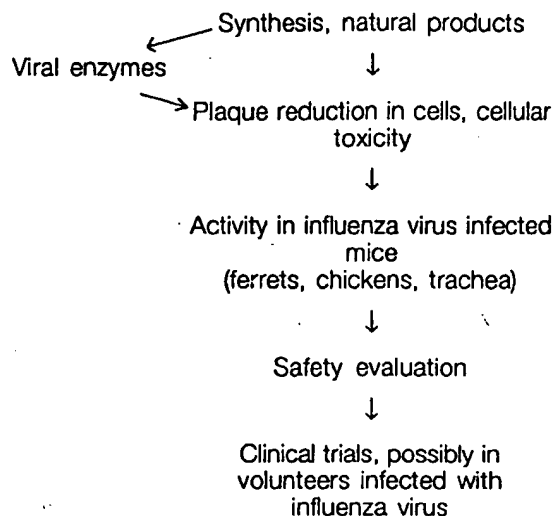


Figure 1: Screening for drugs for treatment of influenza.

by rhinoviruses in humans makes it feasible to infect and treat volunteers once the safety of the drug has been determined in animals (7). Several drugs have been evaluated in infected volunteers, and a correlation between cell culture effects and clinical efficacy has been observed in prophylaxis trials (7).

The rapid course of a rhinovirus infection is likely to limit any antiviral therapy since viral replication will be largely completed when symptoms appear and treatment can be started. Furthermore, pharmacokinetic properties of tested compounds have not been favourable at a high concentration in nasal secretions (7).

Influenza Viruses

There are three anti-influenza drugs with clinical efficacy: amantadine, rimantadine and ribavirin. Reduction of influenza plaque formation in cells has been used as a primary screening system, as outlined in Figure 1, but viral enzymes can also be used. In the cell culture assay amantadine, rimantadine and ribavirin have all showed activity. The assay can identify influenza A mutants resistant to amantadine and rimantadine, the lack of activity of these two drugs against influenza B correlating to a lack of clinical efficacy against infections with influenza B virus (3). Screening of anti-influenza compounds in mice infected with mouse-adopted influenza virus shows good activity of amantadine, rimantadine and ribavirin.

Oral treatment of influenza A virus infection in patients with amantadine and rimantadine results in serum levels and nasal secretion levels of the two drugs equal to those required for inhibition in cell cultures (8, 9). Oral treatment of patients with ribavirin does not result in drug levels sufficient to cause inhibition in cell cultures which might explain the lack of clinical efficacy (2, 8). However, aerosol treatment has resulted in clinical efficacy possibly due to a high local concentration (10).

With the two amines, amantadine and rimantadine, cell culture and mouse infection seem to be relevant screening methods. The validity of these models for testing a nucleoside analogue such as ribavirin is less clear, but this type of inhibitor is at least identified by both systems.

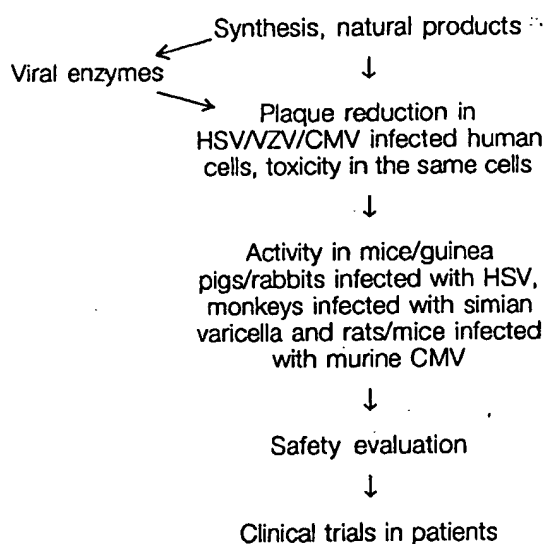


Figure 2: Screening for drugs for treatment of herpesvirus infections.

Herpesviruses

There are six or seven herpes viruses causing disease in humans; of these herpes simplex virus 1 and 2 (HSV-1 and HSV-2), varicella zoster virus (VZV) and cytomegalovirus (CMV) have been the main targets of screening of antiviral drugs. Plaque and immunofluorescence assays in human cells are easy to perform in the case of HSV and CMV, and slightly more difficult in the case of VZV. A general outline of the screening for antiherpes drugs is given in Figure 2.

The difficulty in selecting compounds for clinical evaluation in HSV infections is illustrated in Table 1. In this table the relative efficacy of some antiherpes compounds in cell culture and in different animal models is shown using the same isolate of HSV-1 in all experiments (11). It can be seen that the relative order of efficacy is not the same in cell culture and in animal models and that the results differ between models. To some extent this can be explained by different metabolism of nucleoside analogs in different cells and by differences in pharmacokinetic (11, 12). However, what is rather problematical from a screening point of view is that none of these compounds, nor any other HSV inhibitor, shows more than marginal activity when used for therapy of mucocutaneous HSV infections in humans (13, 14). To some extent this seems to be due to differences in pathogenesis between the primary infection in animals and the recurrent infection in humans. A model of infection in animals with transferred immunity, resembling recurrent infection in humans, seems to give relevant results (15). Oral prophylaxis with acyclovir (ACV) gives excellent clinical results, such prophylaxis being obtained also in infected mice or guinea pigs (13). In guinea pigs the activity of ACV may be underestimated due to a lower rate

Table 1: Relative order of efficacy of different inhibitors in cell culture and animal models of infection using the same isolate of HSV-1. Form of administration is given in brackets. Modified from Öberg and Johansson (11).

Cell culture monkey cell (ID50, μ M)	Cutaneous infection in guinea pigs (topical)	Genital infection in guinea pigs (topical)	Keratitis in rabbits (topical)	Encephalitis in mice (oral)	Systemic infection in mice	
					(i.p.)	(oral)
BVDU (0.03)	Foscarnet	Foscarnet	BVDU	Ara-A	(R)-DHBG	(R)-DHBG
ACV (0.3)	(R)-DHBG	ACV	ACV	ACV	ACV	ACV
(R)-DHBG (4)	ACV	BVDU	(R)-DHBG	(R)-DHBG	Foscarnet	Ara-A
Ara-A (20)	BVDU	(R)-DHBG	Ara-A	Foscarnet		Foscarnet
Foscarnet (30)	Ara-A	Ara-A	Foscarnet			

ACV = acyclovir, Ara-A = adenine arabinoside A,
BVDU = bromovinyldeoxyuridine (R)-DHBG = (R)-9-[3,4-dihydroxybutyl]guanine.

of phosphorylation of ACV to triphosphate than that seen in human cells (16).

The activity of inhibitors against VZV in cell culture seems to correspond to that in monkeys infected with simian varicella (17, 18). The rather low activity of ACV in this monkey model also seems to correspond to the limited activity in VZV infected humans (13), but more compounds have to be evaluated in all the models as well as in humans to determine the validity of the screening systems. Effective treatment of post-herpetic neuralgia remains to be found, but longer treatment might give better results in this troublesome form of zoster.

Two compounds have shown efficacy in treatment of CMV infections: foscarnet (PFA) and ganciclovir (DHPG) (14, 19). Both these compounds show anti-CMV activity in cell cultures, and results in animal models indicate that rat and mouse CMV infection also responds to these drugs (14, 19). Clinical efficacy of foscarnet and ganciclovir has, however, not been seen in all types of CMV infections, and the lack of therapeutic response in bone marrow transplant patients with CMV pneumonitis might be due to pathogenesis involving immune reactions rather than a lytic CMV replication in lung tissue.

Human Immunodeficiency Virus

A number of screening systems are used in the search for drugs against HIV/AIDS. Viral reverse transcriptase, proteinase and other HIV proteins can be used in cell free assays (20), but most primary screening is done in cell cultures utilizing T cells, monocyte and macrophages of primary origin or cell lines (Figure 3). Since HIV replicates in several different types of cells in vivo, the use of one cell type for in vitro screening can be misleading. From a practical point of view screening of a large number of compounds could be done in a T cell line such as H9 cells, but any compound showing activity in that cell should also be evaluated in primary T cells and monocyte/macrophages. In specific structure-activity studies involving a more limited number of compounds parallel evaluation in at least one type of T cell and one monocyte/macrophage seems necessary.

In the primary screening for anti-HIV activity in cell cultures using one isolate of HIV an isolate with typical and well characterized properties, such as HTLV-III_B or HIV_{MN}, should be used. Any active compound should then be evaluated using several HIV isolates, including patient isolates showing resistance to azidothymidine and in the future also isolates showing resistance to other drugs.

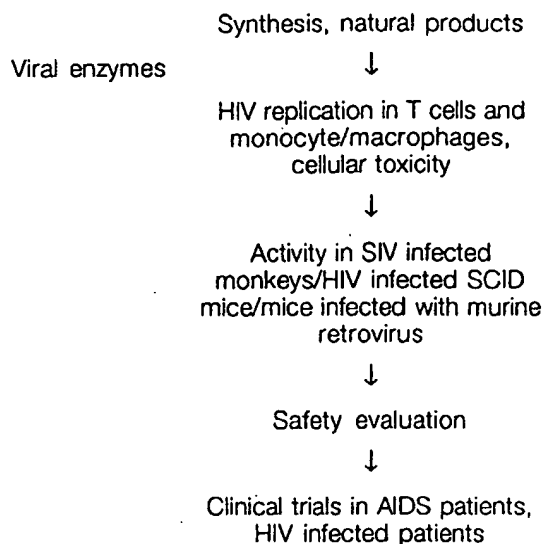


Figure 3: Screening for drugs for treatment of HIV infection and AIDS.

Many different animal models are being evaluated at present for use as secondary screening systems (6, 21, 22). Many of the potential anti-HIV compounds being evaluated are nucleoside analogs (20). It seems especially important to have an animal model with enzymatic properties which corresponds as closely as possible to the human situation. Preliminary results indicate that monkey cells are closer to human cells than mouse cells with respect to phosphorylation of nucleoside analogs (S. Eriksson personal communication).

Infection with simian immunodeficiency virus (SIV) in cynomolgus monkeys has been found to resemble HIV infection in humans (23, 24), and antiviral drugs have shown activity in this model at dose levels which are effective in humans (unpublished observation). The main disadvantages of the SIV infection model in monkeys are the very high costs for the animals, the requirement for monkey P-3 facilities and the need for a substantial amount of drug.

The SCID mouse model (25) offers the possibility of testing a compound against HIV infected human cells in a mouse and has the advantages of the small size of the animal and the use of human cells and virus. However, infection and treatment of HIV infection is limited to the types of human cells transplanted to the mice. Murine retrovirus infections in mice have many advantages when considering costs and handling, but the murine viruses and murine metabolism seem likely to differ too much from HIV and human metabolism to make these systems useful (26).

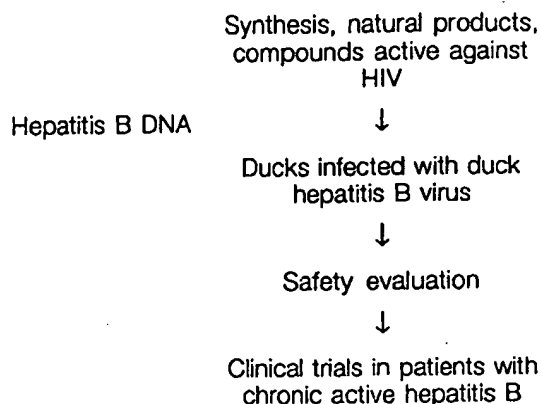


Figure 4: Screening for drugs for treatment of hepatitis B

It can be seen that the ranking of compounds according to antiviral activity in T cell cultures does not reflect the activity in humans. Azidothymidine and dideoxyinosine give 50 % inhibition of HIV in H9 cells at doses of about 0.005 μ M and 0.5 μ M respectively (L. Vrang, unpublished observation) while similar dose levels of these compounds are used in AIDS patients (27, 28).

Hepatitis B Virus

Despite the development of a vaccine against hepatitis B, there is a marked need for drugs, mainly in the treatment of chronic infection. One difficulty in screening for compounds against hepatitis B is the lack of suitable cell culture models. At present primary screening can be done using hepatitis B DNA polymerase (29) and further screening in ducks infected with duck hepatitis B virus (30), as shown in Figure 4.

The similarity in molecular structure of hepatitis B DNA polymerase and HIV reverse transcriptase means that compounds identified as HIV inhibitors could possibly be active against hepatitis B. Compounds active against HIV reverse transcriptase have been tested against hepatitis B DNA polymerase, similar results being obtained (29, 31). However, the nucleoside analogs tested so far might require kinases not present in resting liver cells for their phosphorylation which could be an explanation for the lack of activity of some compounds in ducks infected with duck hepatitis B virus.

A suitable cell culture model for hepatitis B virus would greatly improve the possibility of screening for drugs against this infection. The relevance of such screening systems would need to be demonstrated by relating results to clinical efficacy of one or more drugs.

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